

# Restoration of Silenced Peutz–Jeghers Syndrome Gene, *LKB1*, Induces Apoptosis in Pancreatic Carcinoma Cells<sup>1</sup>

Suparna Qanungo, Subrata Haldar and Aruna Basu

Department of Research, MetroHealth Medical Center, Cleveland, OH 44109, USA

## Abstract

Germ line mutations of the *LKB1* tumor suppressor gene lead to Peutz–Jeghers syndrome (PJS) with a predisposition to cancer. Previous reports suggest that inactivation of this tumor-suppressor gene plays a role in the pathogenesis of gastrointestinal hamartomas as well as several cancers, including adenocarcinoma of the pancreas. Here, we have shown that *LKB1* gene is silenced in the pancreatic cancer cell line AsPC-1, but can be recovered by treatment with the methylation inhibitor, 5-aza-2'-deoxycytidine (5aza2dC). Restoring the level of *LKB1* through gene transfer initiated mitochondria-mediated apoptosis in AsPC-1 cells, as evidenced by the release of cytochrome c from the mitochondria. By confocal microscopy as well as biochemical fractionation, we demonstrate that *LKB1* is present in the nuclear and mitochondrial compartments of pancreatic cancer cells. Our observations also indicate that although functional p53 is absent, the p53 kin, p73, is inducible by doxorubicin in AsPC-1 cells. This suggests that *LKB1*-induced apoptosis is p53 independent but might be p73-mediated in the pancreatic tumor cell line, AsPC-1.

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**Keywords:** *LKB1*; Peutz–Jeghers syndrome; pancreatic cancer; apoptosis.

## Introduction

Peutz–Jeghers syndrome (PJS) is an autosomal dominantly inherited disease [1,2]. Hamartomatous polyps of the gastrointestinal tract and melanin pigmentation of the lips and buccal mucosa characterize this syndrome [1,2]. Patients with PJS have an increased risk of developing cancers of a variety of tissues [3–11]. The causative gene of PJS has been found to be *LKB1/STK11* [7,12]. By linkage analysis, *LKB1/STK11* was mapped to chromosome 19p13.3 [12]. *LKB1* is widely expressed in adult human tissues except in *Xenopus*, where expression of its homologue is restricted to early embryogenesis [13]. The presumptive wild-type allele was noted to be lost in the hamartomas, thus suggesting that *LKB1* is a potential tumor suppressor [4,14]. The human *LKB1* gene encodes a serine/threonine protein kinase that is deficient in the majority of patients with PJS. Besides homozygous

deletion, an alternative mechanism for the inactivation of *LKB1* gene in sporadic neoplasms is promoter hypermethylation [9].

Recently, the mechanism of action of *LKB1* has begun to emerge. It has been shown that *LKB1* is overexpressed in apoptotic cells of the small intestine [15]. Based on this, the apoptosis-inducing ability of *LKB1* was investigated. Interestingly, the ectopic overexpression of *LKB1* induced apoptosis in a kinase-dependent manner in cells harboring functional p53 [15]. On the other end, the overexpression of *LKB1* in *LKB1*-deficient cells was shown to suppress cell growth accompanied by a G<sub>1</sub> cell cycle arrest with the induction of p21 promoter [16]. Both G<sub>1</sub> arrest and the induction of p21 promoter were found to be p53-dependent.

Moreover, recent *in vitro* cloning studies identified Brg1, a component of chromatin remodeling complex, to be an interacting protein of *LKB1*, and *LKB1* kinase activity was shown to be required for Brg1-dependent growth arrest [17]. Besides, studies on *LKB1* knockout mice revealed its role in regulating the expression of the vascular endothelial growth factor, thus suggesting the involvement of *LKB1* in angiogenesis [18]. *LKB1* heterozygous knockout mice developed gastrointestinal polyps resembling the histologic characteristics of PJS [19] as well as hepatocellular carcinoma [5].

In our laboratory, we were primarily interested in analyzing the expression of *LKB1* in pancreatic cancer cells. Our result reveals the presence of *LKB1* in most of the cell lines tested except in a human pancreatic adenocarcinoma cell line, AsPC-1, where *LKB1* is silenced by promoter hypermethylation. Furthermore, we have observed that overexpression of *LKB1* can initiate programmed cell death in a mitochondria-dependent manner in the *LKB1*/p53 null cell line, AsPC-1. We propose that although *LKB1*-induced apoptosis is p53-independent in AsPC-1 cells, it might be regulated by the p53 kin, p73 [20].

Address all correspondence to: Aruna Basu, R455, Rammelkamp Building, MetroHealth Medical Center, 2500 MetroHealth Drive, Cleveland, OH 44109, USA. E-mail: [abasu@metrohealth.org](mailto:abasu@metrohealth.org)

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## Materials and Methods

### Reagents

Goat polyclonal antibody against human *LKB1*, rabbit polyclonal antibody against human p73, and mouse monoclonal antibody against human p53 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Mouse monoclonal  $\beta$ -actin and cytochrome *c* antibodies were obtained from Sigma (St. Louis, MO) and Pharmingen (San Diego, CA), respectively. Cytochrome *c* oxidase subunit I (COX I) antibody was from Molecular Probes (Eugene, OR). Caspase inhibitor z-VAD was purchased from R&D Systems (Minneapolis, MN).

### Cells and Culture Conditions

Human pancreatic cancer cell lines MIA PaCa-2, PANC-1, AsPC-1, BxPC-3, Hs 766T, SU 8686, and CFPAC-1 were grown in RPMI supplemented with 10% FBS and 50  $\mu$ g/ml

gentamicin at 37°C in a 5% CO<sub>2</sub> humidified atmosphere. Transient transfection of the GFP-*LKB1* construct in AsPC-1 cells was performed by calcium phosphate coprecipitation method [21].

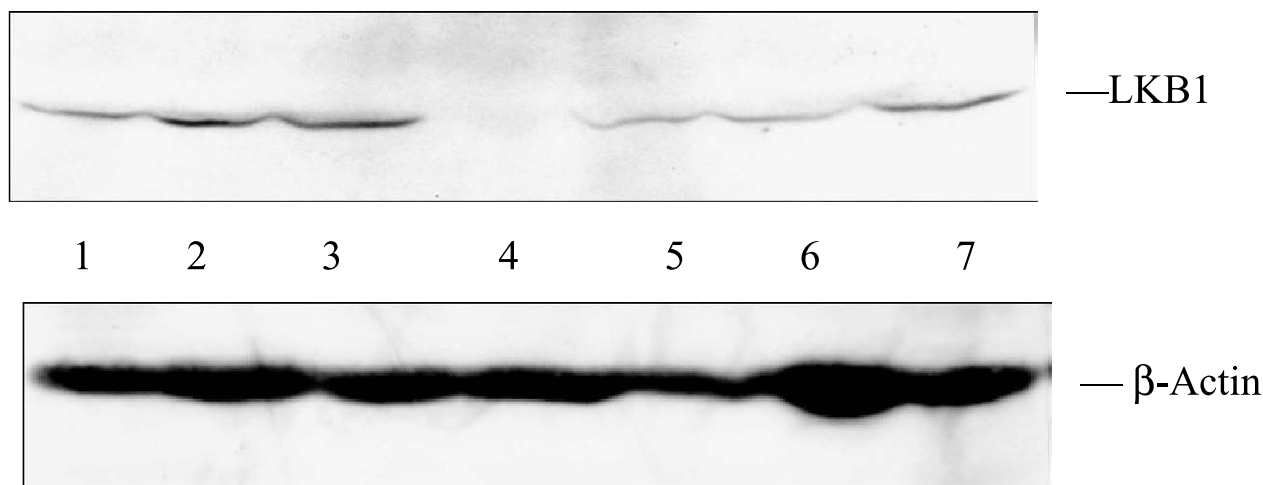
### Assessment of the Level of Expression of *LKB1* and p53 Protein

Total cellular proteins were extracted as described before [22,23]. After normalization for total protein content, the resulting lysate was subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting [22,23]. Immunodetection was accomplished by enhanced chemiluminescence kit available from Amersham/Pharmacia Biotech (Piscataway, NJ).

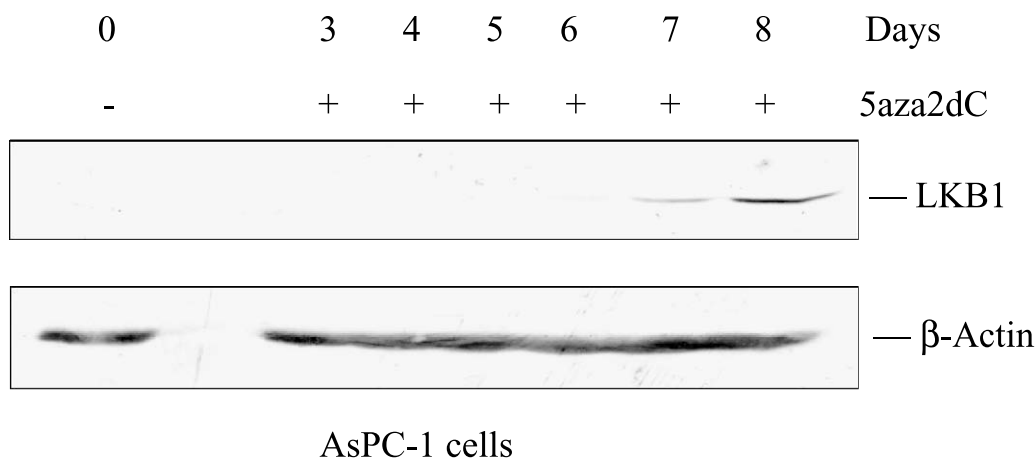
### Subcellular Fractionation

Isolation of a highly enriched mitochondrial fraction as well as cytosolic fraction of GFP-*LKB1*-transfected AsPC-1

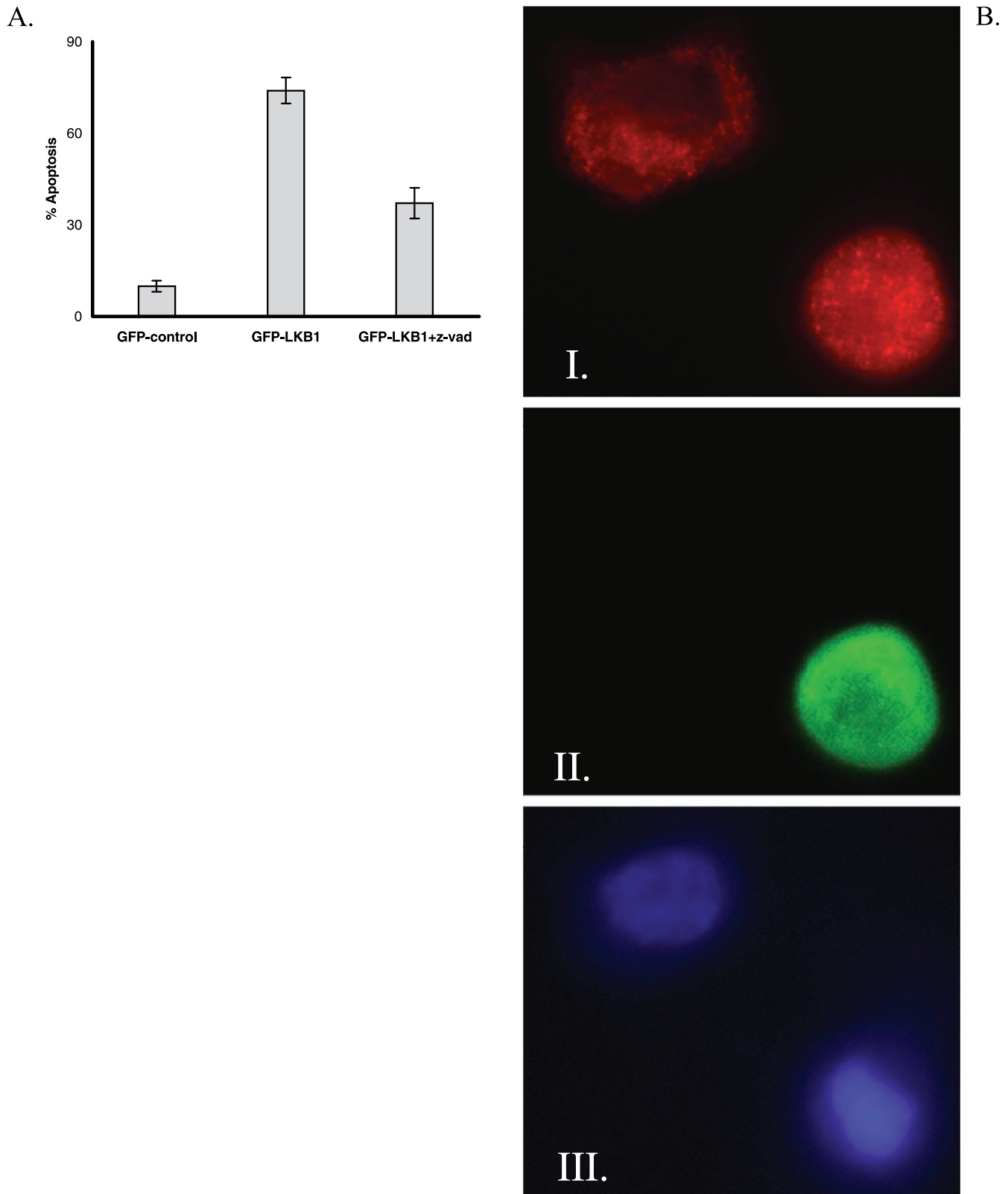
A.



B.



**Figure 1.** Epigenetic inactivation of *LKB1* in pancreatic cancer. Panel A: Loss of *LKB1* in the pancreatic cancer cell line, AsPC-1. Lane 1: MIA PaCa-2; lane 2: BxPC-3; lane 3: SU 8686; lane 4: AsPC-1; lane 5: Hs766T; lane 6: CFPAC-1; and lane 7: PANC-1. Total cellular proteins were isolated followed by fractionation on SDS-PAGE. Proteins were electrophoretically transferred onto a nitrocellulose membrane. Immunoblotting was performed using antibody against human *LKB1*. Panel B: Restoration of *LKB1* in AsPC-1 cells by treatment with the demethylating agent, 5-aza-2'-deoxycytidine. AsPC-1 cells were treated with 3  $\mu$ M 5aza2dC for the indicated period of time. Cellular extracts were immunoblotted with both *LKB1* and  $\beta$ -actin antibodies.



**Figure 2.** Overexpression of LKB1 promotes apoptosis in the LKB1 null cell line, AsPC-1. Panel A: Assessment of apoptosis by DAPI staining. Indicated expression constructs were transfected into AsPC-1 cells. Sixteen hours posttransfection, cells were either treated with 500 nM z-VAD or vehicle solvent and were incubated further a for 24 hours. Cells were fixed with 4% p-formaldehyde followed by morphological determination of apoptosis by DAPI staining. Approximately 200 GFP-positive cells were scored in each test. Percent of apoptosis was calculated as the percent of GFP-positive cells containing condensed nuclei with respect to the total number of cells carrying GFP fluorescence. The results are exhibited as mean  $\pm$  SD. Panel B: Immunofluorescence detection of cytochrome c release from mitochondria in GFP-LKB1-transfected cells. (I) CY3 fluorescence in cells labeled with mouse monoclonal cytochrome c antibody as primary and CY3-conjugated antimouse IgG as secondary. (II) GFP fluorescence pattern in GFP-LKB1-transfected cell. (III) DAPI staining of nuclei of both transfected and untransfected cells.

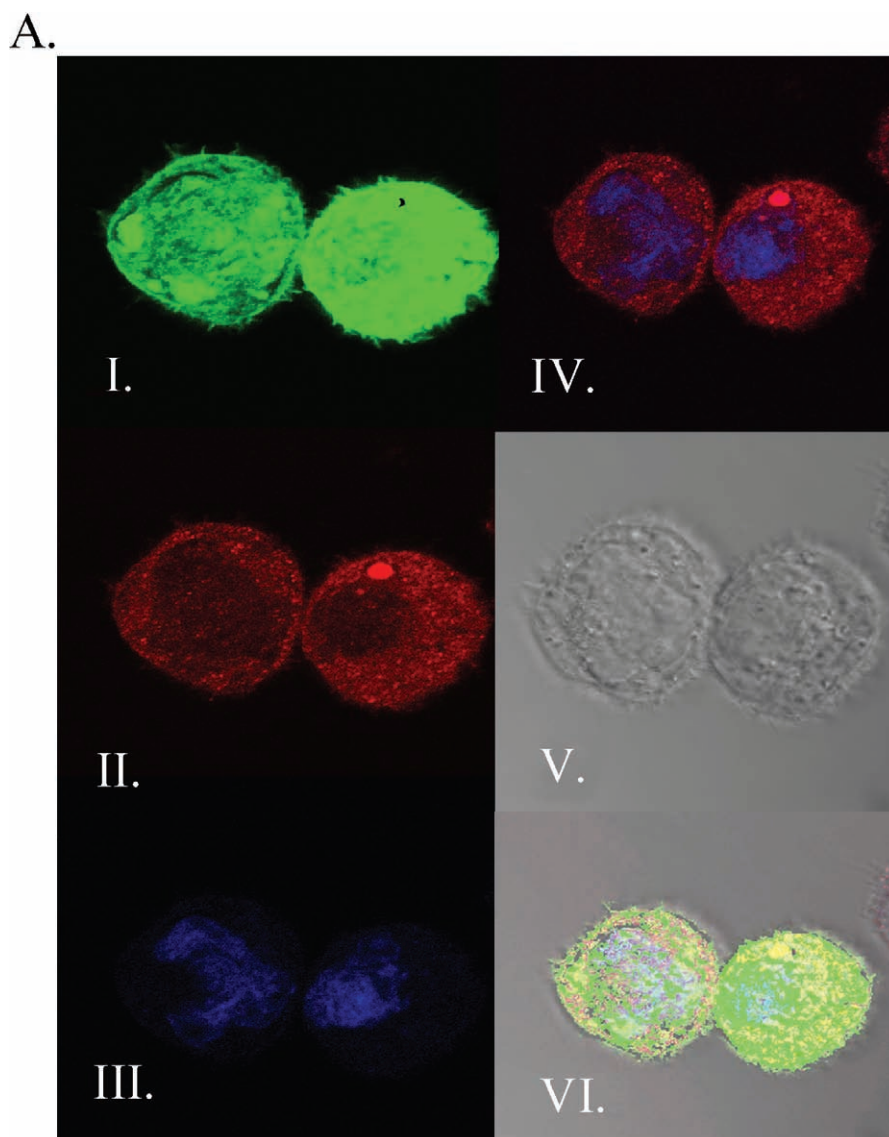
cells was carried out using a kit available from BioVision (Mountain View, CA). Briefly,  $5 \times 10^7$  cells were suspended in  $1 \times$  cytosolic extraction buffer mix containing DTT and protease inhibitors. Following incubation on ice for 10 minutes, cells were homogenized in ice-cold Dounce homogenizer. The homogenate was centrifuged at  $700 \times g$  for 10 minutes at  $4^\circ\text{C}$ . The pellet was collected as crude nuclei. The supernatant of this step was further centrifuged at  $10,000 \times g$  for 30 minutes at  $4^\circ\text{C}$ . The resulting supernatant was collected as cytosol fraction. The pellet was resuspended in mitochondrial extraction buffer containing DTT and protease inhibitors and was saved as mitochondrial fraction.

#### *Analysis of Cell Death*

Chromatin condensation was determined by DAPI fluorescence [22]. Cells were fixed in chilled methanol/acetone (1:1) for 10 minutes at room temperature and permeabilized with 0.5% Tween 20 in PBS for 5 minutes. Subsequently, cells were mounted in a fluid containing  $2 \mu\text{g/ml}$  DAPI (Vector Laboratories, Burlingame, CA). A Nikon (Huntley, IL) Eclipse E600 Fluorescence microscope was used to visualize nuclear stain.

#### *Immunofluorescence Microscopy*

Cells were fixed in 4% paraformaldehyde followed by permeabilization with 0.5% Triton X-100. Blocking was



**Figure 3.** Subcellular localization of ectopically expressed LKB1 in AsPC-1 cells. Panel A: Confocal images of LKB1-transfected AsPC-1 cells. Cells at subconfluent density were transfected with GFP-LKB1. Twenty-four hours after transfection, cells were fixed with 4% p-formaldehyde and processed for immunostaining using mouse monoclonal COX I primary antibody (mitochondrial marker) and CY3-conjugated antimouse secondary antibody. Cells were further stained with TOPRO-3 to visualize nuclei. (I) GFP fluorescence distribution in the cell. (II) COX I staining as evidenced by CY3 fluorescence. (III) TOPRO staining of the nuclei. (IV) Merging of nuclear and mitochondrial staining. (V) Differential interference contrast (DIC) image of the cell. (VI) Merging of GFP fluorescence with the mitochondrial and nuclear stain. Panel B: Western blots of purified mitochondrial fraction from AsPC-1 cells transfected with GFP-LKB1 cDNA. Lane 1: pEGFP-C2 vector-transfected control; and lanes 2 and 3: GFP-LKB1-transfected. Lanes 1 and 2 were probed with anti-GFP antibody and lane 3 with anti-LKB1 antibody. Panel C: Western blots of cytosol and nuclear extracts of LKB1-transfected AsPC-1 cells. Lanes 1 and 3: vector control; and lanes 2 and 4: GFP-LKB1-transfected.

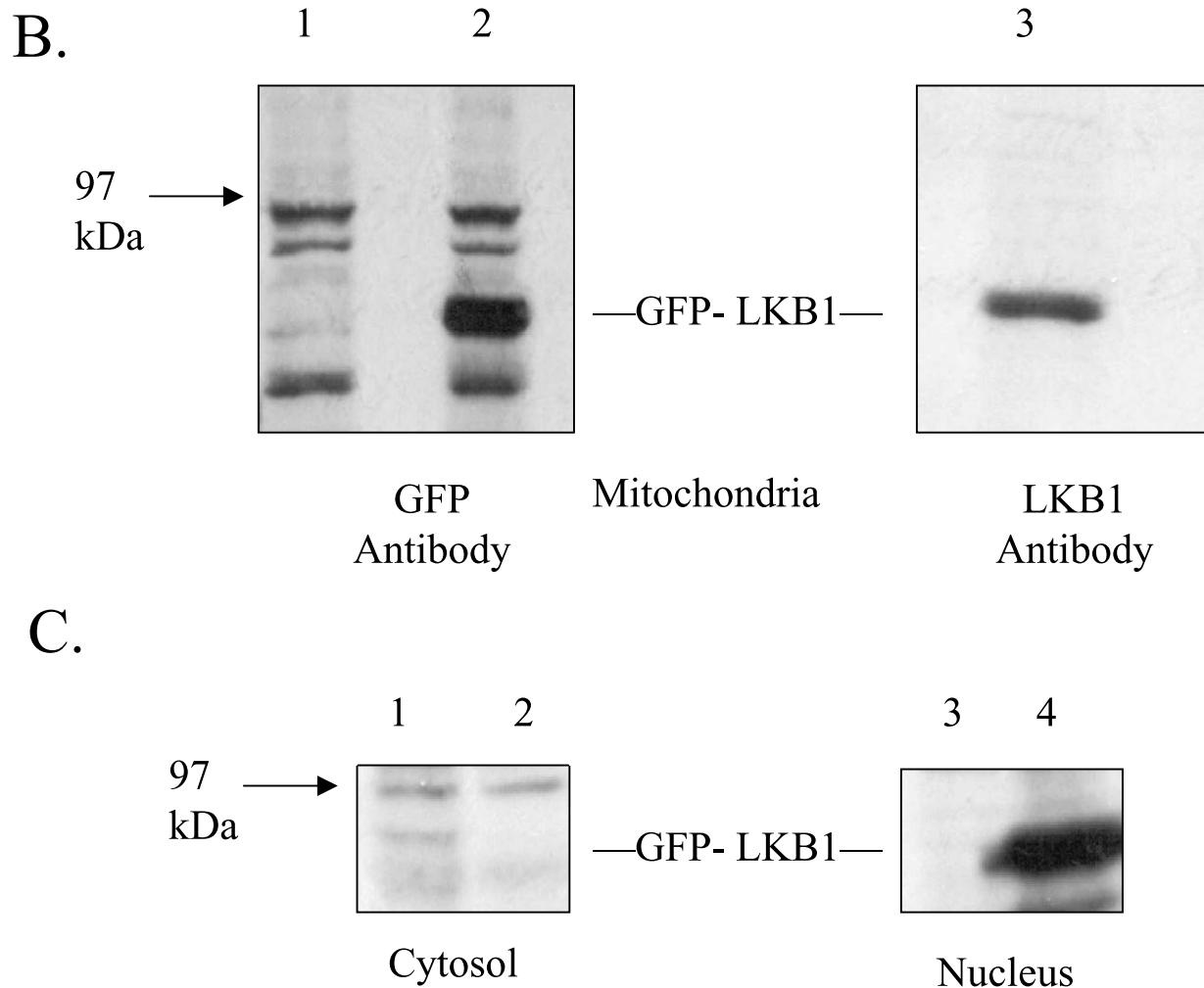


Figure 3. (continued)

done with 4% normal goat serum. Next, the slides were incubated in either mouse monoclonal cytochrome *c* or COX I antibody. Following brief washes, the cells were incubated with CY3-conjugated antimouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA). Subsequently, cells were washed and stained with TOPRO-3 (Molecular Probes, Eugene, OR). Images were captured on either SP2 Leica Confocal microscope (Bannockburn, IL) or Nikon Eclipse E600 fluorescence microscope (Huntley, IL).

## Results

### *Epigenetic Inactivation of LKB1 in the Human Pancreatic Cancer Cell Line, AsPC-1*

Initially, we were interested in testing whether cell lines derived from human pancreatic cancer express *LKB1*. As evident in lane 4 of Figure 1A, of seven pancreatic cancer cell lines tested, the expression of *LKB1* is lost in the human pancreatic adenocarcinoma line, AsPC-1. The loss of expression of *LKB1* by methylation in cancer cells has been reported in the literature [9]. In order to understand whether *LKB1* is silenced by methylation in AsPC-1 cells, we inves-

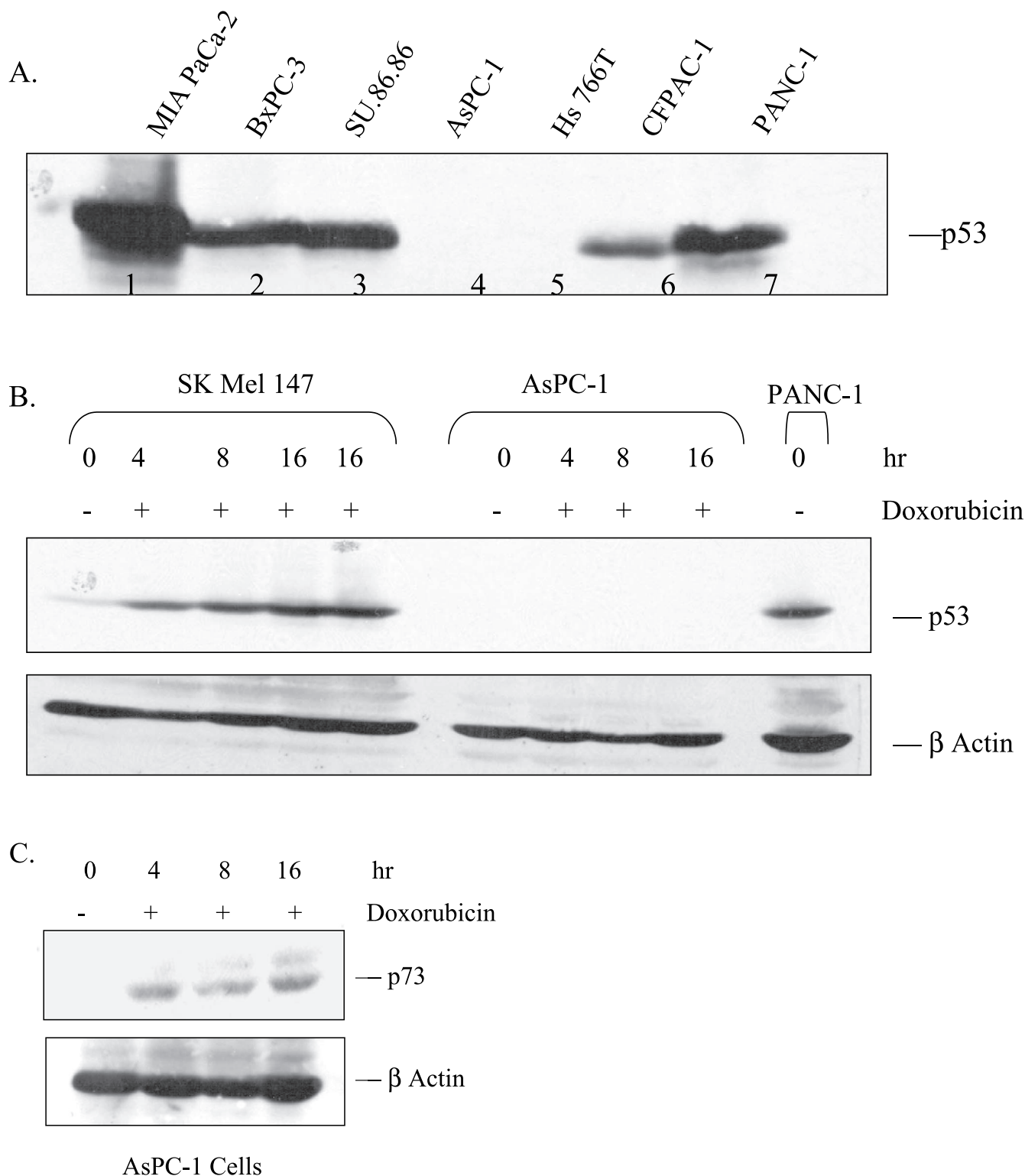
tigated the effect of treatment with demethylating agents such as 5-aza-2'-deoxycytidine (5aza2dC). The treatment of 3  $\mu$ M 5aza2dC for a period of 7 to 8 days can restore the expression of *LKB1* (Figure 1B), indicating the possibility of inactivation of the *LKB1* gene by promoter hypermethylation in AsPC-1 cells.

### *LKB1-Mediated Apoptosis is Mitochondria-Dependent*

The involvement of *LKB1* in the induction of apoptosis has been previously implicated in fibrosarcoma cell lines expressing functional p53 [15]. Here, we were interested in understanding the consequences of *LKB1* overexpression in AsPC-1 cells harboring silenced *LKB1* genes. For this purpose, AsPC-1 cells were transfected with either GFP control vector or GFP-tagged *LKB1* expression constructs. Indeed, overexpression of *LKB1* induced significant cell death in contrast to vector control. Approximately 75% of GFP-positive cells demonstrated the presence of condensed nuclei as evident from DAPI staining. In order to ascertain that *LKB1*-induced apoptosis in pancreatic cancer cells is caspase-dependent, GFP-*LKB1*-transfected cells were treated with a broad-spectrum caspase inhibitor, z-VAD, 16 hours posttransfection. Due

to z-VAD treatment, the extent of GFP-*LKB1*-transfected cells exhibiting nuclear condensation was significantly reduced (Figure 2A).

Mitochondria serve as an important sensor and amplifier in intracellular death signaling pathways. Cytochrome *c* is normally sequestered in the mitochondria and is



**Figure 4.** Functional p73, but not functional or mutant p53, is present in AsPC-1 cells. Panel A: Western blot showing p53 level in pancreatic cancer cells. Panel B: Functional p53 cannot be induced in AsPC-1 cells by doxorubicin contrary to SK Mel 147 cells. Metastatic melanoma cells SK Mel 147 containing functional p53 were used as positive control. Both cells were challenged with 0.3  $\mu$ M doxorubicin for indicated time periods. Western blot with p53 antibody that recognizes both wild and mutant forms of p53 is presented. Panel C: Doxorubicin can induce functional p73 in AsPC-1 cells. AsPC-1 cells were treated with 0.3  $\mu$ M doxorubicin for 4 to 16 hours. Cell lysate was subjected to immunoblot analysis with p73 antibody.



released into the cytosol during early apoptosis due to disruption of the outer mitochondrial membrane. To ask whether *LKB1* induces apoptosis in AsPC-1 cells in a mitochondria-dependent manner through cytochrome *c* release, we performed immunofluorescence staining of *LKB1*-transfected cells using cytochrome *c* antibody. The micrographs revealed a typical mitochondrial pattern [24] in control cells (not GFP-positive) due to lack of apoptosis. This pattern is altered to a diffused staining typical of apoptosis [24], implicating the release of cytochrome *c* from mitochondria of GFP-*LKB1*-transfected cells (Figure 2B, Panels I and II).

#### Subcellular Localization of *LKB1*

In order to assess the localization of overexpressed *LKB1*, immunofluorescence microscopy as well as biochemical fractionation studies were undertaken. The confocal images demonstrate the presence of *LKB1* in both nucleus and mitochondria (Figure 3, Panel A: I–VI). The studies using mitochondrial markers such as COX I antibody indicate the colocalization of GFP stain with COX I (Panel A: I and II). The staining of the nucleus with TOPRO-3 demonstrates the appearance of *LKB1* also in the nuclear compartment (Panel A: I–IV). Panel A, VI of Figure 3 clearly shows the merging of nuclear and mitochondrial stains with GFP fluorescence in transfected AsPC-1 cells. Furthermore, biochemical fractionation also reveals the dual localization of GFP-*LKB1* in mitochondria as well as in nuclei (Figure 3, Panels B and C). Apparently, in GFP-*LKB1*-positive cells, which undergo apoptosis, a pool of *LKB1* is translocated to the mitochondria to initiate apoptosis, as observed earlier [15].

#### *LKB1*-Induced Apoptosis in AsPC-1 Cells is p53-Independent But Might Be Regulated by the p53 kin, p73

The mutations in the *p53* tumor suppressor gene are extremely common in most human cancers. *p53* promotes cell cycle arrest or apoptosis in response to cellular damage.

In order to evaluate whether *LKB1* processes p53-dependent or p53-independent death signal in AsPC-1 cells, we initially tested the level of p53 protein in this pancreatic cancer cell line. The Western blot with p53 antibody, which recognizes both mutant and functional forms of p53, failed to detect p53 proteins in AsPC-1 cells. This observation does not necessarily rule out the presence of functional (wild-type) p53 because it is difficult to detect wild-type p53 without induction by doxorubicin [25]. Surprisingly, treatment of AsPC-1 cells with doxorubicin was without any effect on induction of p53 (Figure 4, Panel A) in contrary to the functional p53-positive melanoma cell line, SK Mel 147 (Figure 4, Panel B).

Because previous observation suggested that the presence of functional p53 is critical in determining *LKB1*-mediated apoptotic response, we thought perhaps a functional p53 homologue like p73 might be involved in AsPC-1 cells to augment apoptosis by *LKB1*. Indeed, p73 was inducible in AsPC-1 cells following doxorubicin treatment (Figure 4, Panel C). Based on this, we propose that

p73, the kin of p53, might be required to activate *LKB1*-mediated cell death in AsPC-1 cells.

#### Discussion

To summarize, our investigation establishes the role of *LKB1* in apoptosis using the *LKB1* null pancreatic cancer cell as a model. The enforced expression of *LKB1* can induce apoptosis in AsPC-1 cells in a mitochondria-dependent pathway through cytochrome *c* release. Apparently, the interaction between the p53 homologue, p73, and *LKB1* might facilitate programmed cell death in this cell line lacking p53. p73 bears significant sequence and functional similarities to p53. p73 isoform is capable of activating p53-responsive genes such as *WAF1/Kip1* and *Bax*; therefore, like p53, it can regulate growth arrest and cell death. p73 might also be expected to have tumor-suppressor function and can substitute for p53, when it is deleted. Clearly, further investigation to demonstrate the interaction of p73 and *LKB1* in AsPC-1 cells is warranted. Nonetheless, our initial observation reporting the ability of *LKB1* gene to permit pancreatic adenocarcinoma cells to die by apoptosis is quite important with respect to its clinical significance. Our studies are concordant with the previous reports describing the reduced extent of apoptotic cells in polyps arising from Peutz–Jegher patients devoid of *LKB1* expression.

Cancer of the pancreas stands out as a highly lethal disease with the poorest likelihood of survival among all of the major malignancies. The development of malignancies is a multistep process that comprises initiation, progression, invasion, and, finally, establishment of metastasis [26,27]. The multiple genetic alterations at each stage impart selective advantage over normal counterpart. In this respect, pancreatic carcinoma is no exception [28,29]. During the last decade, several cancer-causing genes including *LKB1/STK11* and *SMAD4/DPC4* have been found to be associated with pancreatic carcinoma [30]. *SMAD4*, the cytoplasmic messenger of the TGF- $\beta$  signal, can associate with *LKB1* through an *LKB1*-interacting protein, LIP1 [31]. It has been previously reported that the inhibition of *LKB1* can suppress TGF- $\beta$  signaling. It remains to be seen whether inactivation of *LKB1* in the pancreatic cancer cell line, AsPC-1, leads to the down-regulation of TGF- $\beta$  signal, causing suppression of growth inhibition and apoptosis. Interestingly, AsPC-1 cells are insensitive to apoptosis in response to the tubulin-binding drug, 2-methoxyestradiol (Qanungo and Basu, unpublished observation) [32]. Thus, the function of *LKB1* in inducing apoptosis in a subpopulation of pancreatic cancer cells may uphold the future promise for potential therapeutic advantage.

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## References

- [1] Peutz JL (1921). On a very remarkable case of familial polyposis of the mucous membranes of the intestinal tract and nasopharynx accompanied by peculiar pigmentation of the skin and mucous membrane. *Ned Tijdschr Geneesk* **10**, 134–36.
- [2] Jeghers H, McKusick VA, and Karz KH (1949). Generalized intestinal polyposis and melanin spots of the oral mucosa, lips and digits: a syndrome of diagnostic significance. *N Engl J Med* **241**, 992–1005.
- [3] Sanchez-Cespedes M, Parrella P, Esteller M, Nomoto S, Trink B, Engles JM, Westra WH, Herman JG, and Sidransky D (2002). Inactivation of *LKB1/STK11* is a common event in adenocarcinomas of the lung. *Cancer Res* **62**, 3659–662.
- [4] Shen Z, Wen XF, Lan F, Shen ZZ, and Shao ZM (2002). The tumor suppressor gene *LKB1* is associated with prognosis in human breast carcinoma. *Clin Cancer Res* **8**, 2085–2090.
- [5] Nakau M, Miyoshi H, Seldin MF, Imamura M, Oshima M, and Taketo MM (2002). Hepatocellular carcinoma caused by loss of heterozygosity in *Lkb1* gene knockout mice. *Cancer Res* **62**, 4549–553.
- [6] Bardeesy N, Sinha M, Hezel AF, Signoretti S, Hathaway NA, Sharpless NE, Loda M, Carrasco DR, and DePinho RA (2002). Loss of the *Lkb1* tumour suppressor provokes intestinal polyposis but resistance to transformation. *Nature* **419**, 162–67.
- [7] Jishage K, Nezu J, Kawase Y, Iwata T, Watanabe M, Miyoshi A, Ose A, Habu K, Kake T, Kamada N, Ueda O, Kinoshita M, Jenne DE, Shimane M, and Suzuki H (2002). Role of *Lkb1*, the causative gene of Peutz–Jegher's syndrome, in embryogenesis and polyposis. *Proc Natl Acad Sci USA* **99**, 8903–908.
- [8] Miyoshi H, Nakau M, Ishikawa TO, Seldin MF, Oshima M, and Taketo MM (2002). Gastrointestinal hamartomatous polyposis in *Lkb1* heterozygous knockout mice. *Cancer Res* **62**, 2261–266.
- [9] Esteller M, Avizienyte E, Corn PG, Lothe RA, Baylin SB, Aaltonen LA, and Herman JG (2000). Epigenetic inactivation of *LKB1* in primary tumors associated with the Peutz–Jeghers syndrome. *Oncogene* **19**, 164–68.
- [10] Su GH, Hruban RH, Bansal RK, Bova GS, Tang DJ, Shekher MC, Westerman AM, Entius MM, Goggins M, Yeo CJ, and Kern SE (1999). Germ line and somatic mutations of the *STK11/LKB1* Peutz–Jeghers gene in pancreatic and biliary cancers. *Am J Pathol* **154**, 1835–840.
- [11] Wang ZJ, Churchman M, Avizienyte E, McKeown C, Davies S, Evans DG, Ferguson A, Ellis I, Xu WH, Yan ZY, Aaltonen LA, and Tomlinson IP (1999). Germ line mutations of the *LKB1 (STK11)* gene in Peutz–Jeghers patients. *J Med Genet* **36**, 365–68.
- [12] Hemminki A, Tomlinson I, Markie D, Jarvinen H, Sistonen P, Bjorkqvist AM, Knuutila S, Salovaara R, Bodmer W, Shibata D, de la Chapelle A, and Aaltonen LA (1997). Localization of a susceptibility locus for Peutz–Jeghers syndrome to 19p using comparative genomic hybridization and targeted linkage analysis. *Nat Genet* **15**, 87–90.
- [13] Su J, Erickson E, and Maller J (1996). Cloning and characterization of a novel serine/threonine protein kinase expressed in early *Xenopus* embryos. *J Biol Chem* **271**, 14430–437.
- [14] Yoo LI, Chung DC, and Yuan J (2002). *LKB1*—a master tumour suppressor of the small intestine and beyond. *Nat Rev Cancer* **2**, 529–35.
- [15] Karuman P, Gozani O, Odze RD, Zhou XC, Zhu H, Shaw R, Brien TP, Bozzuto CD, Ooi D, Cantley LC, and Yuan J (2001). The Peutz–Jegher gene product *LKB1* is a mediator of p53-dependent cell death. *Mol Cell* **7**, 1307–319.
- [16] Tiainen M, Vaahtomeri K, Ylikorkala A, and Makela TP (2002). Growth arrest by the *LKB1* tumor suppressor: induction of p21 (WAF1/CIP1). *Hum Mol Genet* **11**, 497–504.
- [17] Marignani PA, Kanai F, and Carpenter CL (2001). *LKB1* associates with Brg1 and is necessary for Brg1-induced growth arrest. *J Biol Chem* **276**, 32415–418.
- [18] Ylikorkala A, Rossi DJ, Korsisaari N, Luukko K, Alitalo K, Henkemeyer M, and Makela TP (2001). Vascular abnormalities and deregulation of VEGF in *Lkb1*-deficient mice. *Science* **293**, 323–326.
- [19] Miyoshi H, Nakau M, Ishikawa TO, Seldin MF, Oshima M, and Taketo MM (2002). Gastrointestinal hamartomatous polyposis in *Lkb1* heterozygous knockout mice. *Cancer Res* **62**, 2261–266.
- [20] Melino G, De Laurenzi V, and Vousden KH (2002). p73: friend or foe in tumorigenesis. *Nat Rev Cancer* **2**, 605–15.
- [21] Haldar S, Basu A, and Croce CM (1998). Serine-70 is one of the critical sites for drug-induced Bcl2 phosphorylation in cancer cells. *Cancer Res* **58**, 1609–615.
- [22] Basu A, Das M, Qanungo S, Fan XJ, DuBois G, and Haldar S (2002). Proteasomal degradation of human peptidyl prolyl isomerase pin-pointing phospho Bcl2 toward dephosphorylation. *Neoplasia* **4**, 218–27.
- [23] Basu A, and Haldar S (2003). Identification of a novel Bcl-xL phosphorylation site regulating the sensitivity of taxol- or 2-methoxyestradiol-induced apoptosis. *FEBS Lett* **538**, 41–47.
- [24] Roberg K, Johansson U, and Ollinger K (1999). Lysosomal release of cathepsin D precedes relocation of cytochrome c and loss of mitochondrial transmembrane potential during apoptosis induced by oxidative stress. *Free Radic Biol Med* **27**, 1228–237.
- [25] Soengas M, Capodieci P, Polsky D, Mora J, Esteller M, Opitz-Araya X, McCombie R, Herman JG, Gerald WL, Lazebnik YA, Cordon-Cardo C, and Lowe SW (2001). Inactivation of the apoptosis effector Apaf-1 inn malignant melanoma. *Nature* **409**, 207–11.
- [26] Han H, Bearss DJ, Browne LW, Calaluze R, Nagle RB, and Von Hoff DD (2002). Identification of differentially expressed genes in pancreatic cancer cells using cDNA microarray. *Cancer Res* **62**, 2890–896.
- [27] Mauro MJ, and Druker BJ (2001). STI571: a gene product-targeted therapy for leukemia. *Curr Oncol Rep* **3**, 223–27.
- [28] Kern SE (2000). Molecular genetic alterations in ductal pancreatic adenocarcinomas. *Med Clin North Am* **84**, 691–95.
- [29] Goggins M, Kern SE, Offerhaus JA, and Hruban RH (1999). Progress in cancer genetics: lessons from pancreatic cancer. *Ann Oncol* **10**, 4–8.
- [30] Sakorafas GH, Tsiotou AG, and Tsiotos GG (2000). Molecular biology of pancreatic cancer; oncogenes, tumor suppressor genes, growth factors and their receptors from a clinical perspective. *Cancer Treat Rev* **26**, 29–52.
- [31] Smith DP, Rayter SI, Niederlander C, Spicer J, Jones CM, and Ashworth A (2001). LIP1, a cytoplasmic protein functionally linked to the Peutz–Jeghers syndrome kinase *LKB1*. *Hum Mol Genet* **10**, 2869–877.
- [32] Qanungo S, Basu A, Das M, and Haldar S (2002). 2-Methoxyestradiol induces mitochondria dependent apoptotic signaling in pancreatic cancer cells. *Oncogene* **21**, 4149–157.